Patients in heart failure are sometimes implanted with a left ventricular assist device (LVAD) to bridge them to transplant. Clinical data shows that ~15% of these patients recover pump function and can subsequently have their LVAD removed. The goal of our study was to determine whether cellular level maximal power output of the left ventricle improves after patients are fitted with LVADs. Myocardial samples were obtained (1) from the left ventricle apex of patients when they were implanted with a LVAD and (2) from the left ventricle free wall when they subsequently received a heart transplant. Non failing cardiac samples were also obtained from organ donors for comparison. All samples were snap frozen in liquid nitrogen and stored in the vapor phase of liquid nitrogen. Multicellular preparations were then obtained by chemically permeabilizing the samples in 1% Triton solution after mechanical homogenization. These preparations were connected between a force transducer and a motor and maximally activated in a saturating Ca^{2+} solution. Once force had reached steady state, the preparations were allowed to shorten against pre-set loads imposed using SLControl software. Maximum power output was determined from the force-velocity curves. Preliminary results suggest that LVAD treatment improved cellular level maximum power output and isometric force. There was also a trend towards a lower maximum shortening velocity post-LVAD. Further studies, will determine whether intrinsic ventricular function is improved by LVAD implantation as well as the molecular mechanisms that may mediate any observed responses.

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The Affect of Omecamtiv Mecarbilon the Phosphoate Dissociation and Motile Properties of the Recombinant Human β-Cardiac Heavymeromyosin

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Omecamtiv mecarbil (OM), a small-molecule increases cardiac contractility by directly activating cardiac myosin by accelerating phosphate (Pi) release (Malik et al., 2011). Here we analyzed the affect of OM on the mechanochemical cycle and motility of human β-cardiac heavymeromyosin expressed in a adenoviral/C2C12 muscle cell based system, and purified porcine ventricular myosin (PV-HMM). Double-mixing stopped-flow fluorescence and phosphate binding protein (MDCC-PBP) were used to determine the maximum rate (k_{max}) of phosphate release. Rates of $30s^{-1}$ and $35s^{-1}$ were obtained for human and porcine cardiac HMM respectively. Addition of 20 μM OM resulted in a 2-fold increase in the maximal rate of actin activated Pi release to k_{max} = 70 s^{-1} (AM-ADP-Pi _> AM-ADP + Pi) for both human and porcine cardiac HMM. The unloaded actin filament velocity measured in the *in vitro* motility assay was 0.76 ± 0.37 μm/sec for human β-cardiac HMM and 0.97 ± 0.23 μm/sec for PV-HMM. OM has two effects on the motor activity: 1) The actin filament velocity was significantly slower (~ 16 -fold at saturation); 2) the filament motion became more persistent with long periods of uninterrupted movement. This suggests that OM recruits more crossbridges to participate in the movement of the actin filaments resulting in higher forces per filament, which overcomes the pinning defects that invariably trap actin filaments in normal motility assays. The increase in attached crossbridges/filament also slows the filament velocity. The motility data are consistent with the kinetic analysis indicating that OM accelerates Pi release without dramatically enhancing the rate of the ATPase hydrolysis. This produces a higher duty ratio in which the motor spends more of the ATPase cycle in tight binding states. Supported by AHA-BGIA to EF and AHA-GI to DAW.

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Impaired Cross Bridge Formation Contributes to Reduced Cardiac Contraction in the Infarct Border Zone

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After myocardial infarction, adjacent to the infarct there is a poorly contracting non-ischemic border zone. The mechanism of border zone dysfunction is unclear. We found that border zone dysfunction does not involve myocardial fibrosis, or a decreased content of either myofibrils or myosin, suggesting there is a defect in the contractile mechanism. **Goal:** Determine the mechanism for border zone dysfunction. **Methods:** We used sheep hearts 2 weeks after apical infarction. Cardiac muscle strips were dissected from the border zone adjacent to the infarct and from a zone remote from the infarct. Myofilament contraction was assessed using *in-vitro* isometric and isotonic contractions of demembranated cardiac muscle strips bathed in activating solutions. To assess strongly bound cross-bridge formation, we measured muscle stiffness using high frequency, low amplitude oscillations of muscle length in the absence of ATP (in this rigor state, cross bridge formation should be maximal). **Results:** In border zone myocardium, maximal force development (Fmax) was reduced by 31 ± 2% (n=6, $P<0.01$) compared to Fmax of remote zone myocardium (85 ± 1 mN/mm^2). The stiffness in the rigor state was reduced by 34 ± 6% (n=5, $P<0.05$) in border zone myocardium versus remote zone myocardium, suggesting impaired cross bridge formation in the border zone. There was no difference between border zone and remote zone myocardium in the maximum velocity of muscle shortening (~0.35 muscle lengths/s), or in the rate constant of force redevelopment (Ktr, ~2.5 s^{-1}) after briefly mechanically disrupting cross bridges with a rapid perturbation of muscle length. **Conclusions:** Impaired contraction of border zone myocardium involves a reduction in cross bridge formation, without effects on cross bridge kinetics. Preliminary studies suggest proteolytic cleavage of contractile proteins may play a role.

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Superinhibitory Phospholemman Mutants as Potential Therapeutics for Heart Failure

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Heart failure is characterized by a decrease in cardiac contractility and is a leading cause of morbidity and mortality. Treatment with cardiac glycosides such as digitalis increases cardiac contractility by elevating intracellular calcium through inhibition of the sodium-potassium ATPase (NKA). However, the therapeutic window for this class of drugs is narrow as high calcium levels lead to increased SR load, calcium leak, and cardiac arrhythmias. Alternatively, the endogenous inhibitor of NKA, phospholemman (PLM), is dynamically regulated. We hypothesize that a superinhibitory mutant of PLM will increase the contractility of cardiac myocytes. This effect will be inherently self-limiting since inhibition of NKA is relieved by phosphorylation or elevated intracellular sodium. Here we used a high-throughput screen of PLM mutants utilizing fluorescence microscopy to simultaneously measure PLM-NKA and PLM-PLM binding. By tagging NKA with CFP and PLM with YFP, we can measure NKA-PLM binding with heterotransfer FRET (CFP to YFP) and oligomerization of PLM with homotransfer FRET (YFP to YFP). We observed that several mutants have a decreased affinity for oligomerization (PLM-PLM binding) leading to an increased apparent affinity for NKA. Future plans include creating and screening many more mutants and measuring the functional effect of PLM-based superinhibitors in vitro and in vivo.

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Electric-Field-Based Control Strategies for Cardiac Tissue

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Ventricular fibrillation is one of the leading causes of death in the world. Current therapy is based on a high-energy electric shock, which indiscriminately excites every cell in the muscle and may cause severe side effects such as tissue damage and intolerable pain. The complex spatio-temporal dynamics of fibrillation, induced by multiple interacting spiral waves, makes the design of robust low-energy control strategies a challenging scientific problem.
Low-energy electric field stimuli lead to the formation of wave emitting sites at structural heterogeneities in the tissue, with a density adjustable through field strength. By applying a sequence of electric-field pulses, Low-Energy Anti-Fibrillation pacing (LEAP) uses these wave sources to non-invasively control the tissue from multiple sites and terminate fibrillation by progressive synchronization. We present experiments on canine dogs, investigating the application of LEAP to atrial (in-vitro and in-vivo) and ventricular fibrillation (in-vitro) as well as the wave source recruitment mechanism in quiescent tissue [1]. We show that the observed wave source density is in agreement with theoretical predictions based on the size distribution of the cardiovascular tree. These findings are supported and extended by theoretical investigations revealing a general curvature-dependent sensitivity of anatomical features to electric-field stimulation [2]. LEAP results in an energy reduction of 80% for the termination of fibrillation compared to conventional defibrillation. Further energy reduction might be possible in specific scenarios by making use of negative-curvature structures on the endocardium such as trabecular, papillary and pectinate muscles.
References:
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